Widespread Decrease of Nicotinic Acetylcholine Receptors in Parkinson's Disease

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Objective: **Nicotinic acetylcholine receptors have close interactions with the dopaminergic system and play critical roles in cognitive function. The purpose of this study was to compare these receptors between living PD patients and healthy subjects.** ***Methods:*** **Nicotinic acetylcholine receptors were imaged in 10 nondemented Parkinson's disease patients and 15 age-matched healthy subjects using a single-photon emission computed tomography ligand [^{123}I]5-iodo-3-[2(*S*)-2-azetidinylmethoxy]pyridine. Using an arterial input function, we measured the total distribution volume (*V;* specific plus nondisplaceable), as well as the delivery (K_1).** ***Results:*** **Parkinson's disease showed a widespread significant decrease (approximately 10%) of *V* in both cortical and subcortical regions without a significant change in K_1.** ***Interpretation:*** **These results indicate the importance of extending the study to demented patients.**

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In addition to the well-documented loss of dopaminergic neurons, a number of animal and clinical studies have shown that nicotinic acetylcholine receptors (nAChRs) play critical roles in Parkinson's disease (PD). nAChR activation stimulates dopamine release in the striatum,[1] and an agonist at nAChRs showed synergistic therapeutic effects with L-dopa in a monkey model of PD.[2] Epidemiological studies showed that cigarette smoking protects against PD.[3] Both animal and human studies have shown that nAChR is one of the central components in cognitive function,[4] and a substantial number of PD patients become demented. Finally, most postmortem studies showed widespread decrease of nAChRs both in striatum and cerebral cortices of PD patients.[5–8] However, as in most other postmortem studies, many of these lacked critical clinical information such as the presence of dementia and a history of cigarette smoking. Therefore, it is critical to image nAChRs in living PD patients whose clinical information is available to study changes and to explore possible therapeutic intervention at these receptors. However, to our knowledge, such a study has not been published.

Recently, 3-[2(*S*)-2-azetidinylmethoxy]pyridine (A-85380) has been developed,[9] which has high affinity to the predominant type of nAChRs in the brain composed of α_4 and β_2 subunits.[10] A few analogs of A-85380, including [^{123}I]5-iodo-3-[2(*S*)-2-azetidinylmethoxy]pyridine (5-I-A-85380), have been radiolabeled and used successfully in humans.[11,12] An ex vivo study in nonhuman primate has shown that radiolabeled metabolites of [^{123}I] 5-I-A-85380 do not cross the blood–brain barrier.[13] 5-I-A-85380 labels several β_2-containing nAChRs, including $\alpha_4\beta_2$- (the most predominant nicotinic receptor in human brain), $\alpha_3\beta_2$-, and $\alpha6\beta2$-containing subtypes.[14]

The purpose of this study was to perform a pilot study of nAChR imaging using [^{123}I]5-I-A-85380 in early to moderate stage PD patients.

Subjects and Methods

Subjects

The study was approved by National Institute of Neurological Disorders and Stroke and National Institute of Mental Health institutional review boards. Patients were recruited from National Institute of Neurological Disorders and Stroke clinics. Control subjects were healthy volunteers recruited from community via advertisement who did not have a history or signs of neurological disorders. After complete description of the study to the subjects, written informed consent was obtained. Sample demographics and clinical characteristics are shown in Table 1. For all participants, the absence of axial focal lesions was confirmed by a neuroradiologist using noncontrast magnetic resonance imaging. All patients and healthy subjects had not smoked cigarettes for at least 5 years. None of the patients had used cholinergic or anticholinergic medications within 60 days of the single-photon emission computed tomography (SPECT) scan. On the day of the SPECT scans, all patients continued dopaminergic medications including carbidopa/L-dopa. There was no significant difference in age between PD patients and healthy subjects.

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Table 1. Sample Demographics and Clinical Characteristics

Characteristics	Healthy	PD
N	15	10
Mean age (±SD), yr	59 ± 5	56 ± 3
Sex, F/M	10/5	4/6
Cigarette smoking	No	No
Cholinergic medication	No	No
Dopaminergic medication	No	Yes
Mean [^{123}I]5-I-A-85380 dose (±SD), MBq	486 ± 79	506 ± 75
Mean Hoehn and Yahr staging (±SD)		2.5 ± 0.4
Mean total Unified Parkinson's Disease Rating Scale score (±SD)		46 ± 5[a]
Mean Mini-Mental Status Examination score		≥27
Mean Mattis Dementia Rating Scale score (±SD)		135 ± 4[b]

[a]N = 9; [b]N = 8.

PD = Parkinson's disease; SD = standard deviation.

Clinical Ratings

Motor function was evaluated by Unified Parkinson's Disease Rating Scale and Hoehn and Yahr staging. Cognitive function was measured with Mini-Mental Status Examination and Mattis Dementia Rating Scale.

Single-Photon Emission Computed Tomography

[^{123}I]5-I-A-85380 was prepared as described previously.[12] SPECT data were acquired using a triple-headed camera with low-energy, high-resolution, parallel hole collimators (Trionix XLT-20; Triad, Twinsburg, OH). Initially, a transmission scan was obtained using a ^{153}Gd line source. Subsequently, [^{123}I]5-I-A-85380 was administered intravenously as a bolus (injection dose: healthy, 486 ± 79MBq; PD, 506 ± 75MBq; no significant difference, with these and subsequent data expressed as mean ± standard deviation). SPECT data were acquired at 0 to 40, 115 to 135, and 210 to 230 minutes. Arterial samples were obtained every 15 seconds for the first 2 minutes and at 3, 4, 5, 10, 30, 80, 120, and 180 minutes.

Plasma Analysis

Plasma [^{123}I]5-I-A-85380 concentration and the free fraction (f_1) were determined as described previously.[12]

Image Analysis

SPECT projection data were reconstructed on a 64 × 64 matrix with pixel size of 4.48 × 4.48 × 4.48mm in the *x*-, *y*-, and *z*-axis, respectively, with correction for attenuation and scattered radiation.[15] Parametric images of the delivery of the radioligand (K_1) and total distribution volume *(V)* were created using a multilinear algorithm[16,17] implemented in PMOD 2.55 (http://www.pmod.com/technologies/index.html). Plasma free [^{123}I]5-I-A-85380 levels were used to calculate *V* instead of using plasma total (free plus protein bound) [^{123}I]5-I-A-85380 because patients were taking medication and concomitant medication might change plasma protein binding of [^{123}I]5-I-A-85380. Parametric images were spatially normalized to a standard anatomic orientation (Montreal Neurological Institute space) based on K_1 images and using Statistical Parametric Mapping version '02 (SPM2; http://www.fil.ion.ucl.ac.uk/spm). Spatially normalized K_1 and *V* images were smoothed with 10mm full-width at half-maximum. To confirm the magnitude of changes in *V,* we obtained volume of interest data from brain regions in Montreal Neurological Institute space listed on Table 2.

Statistical Analysis

SPM2[18] was used for statistical analysis. Two-sample *t* test was applied to compare K_1 and *V* between patients and healthy subjects, and simple regression analysis was applied to study the relationship between *V* and clinical ratings. Gray matter threshold was set at 20% for *V* and 80% for K_1 images, respectively. Because each pixel had a measured value of K_1 or *V,* global normalization was not applied. No sphericity correction was applied by assuming replication over groups. False-discovery rate of *p* less than 0.05 and cluster-level corrected *p* less than 0.05 were considered significant.

A two-sample *t* test was applied to compare plasma free fraction of [^{123}I]5-I-A-85380 between groups.

Results

Patients were in the early to moderate stages of PD and were not demented (see Table 1). Patients tended to show lower f_1 values than healthy subjects ($p = 0.09$; see Table 2).

An SPM *t* test showed a significant decrease of *V* in many brain regions with the greatest *T* value of 4.96 (Fig). The decrease measured by the volume of interest was 15% in thalamus, whereas occipital and frontal cortices showed only 3 and 5% decreases, respectively, where many voxels did not reach significance. Decreases in parietal and temporal cortices were 8 to 9% (see Table 2). SPM did not detect a significant increase of *V* in any brain region in patients. There was neither a significant increase nor a decrease of K_1 in any re-

Table 2. Plasma-Free Fraction and Total Distribution Volume of [^{123}I]5-I-A-85380

Measurements	Healthy	PD
Plasma-free fraction, %	48.1 ± 4.4	45.3 ± 3.1
Total distribution volume, ml/cm^3		
Thalamus	71.9 ± 13.5	61.2 ± 10.7
Caudate	41.9 ± 6.2	36.9 ± 4.3
Putamen	44.4 ± 6.7	40.2 ± 5.6
Pons	43.3 ± 8.1	39.0 ± 8.1
Frontal cortex	24.9 ± 3.1	23.7 ± 3.1
Parietal cortex	26.9 ± 3.5	24.4 ± 3.0
Temporal cortex	30.7 ± 3.9	28.1 ± 4.0
Occipital cortex	27.1 ± 3.6	26.3 ± 4.2
Cerebellum	33.8 ± 6.2	29.2 ± 5.9[a]

[a]One patient was excluded whose cerebellum was partially out of field of view.

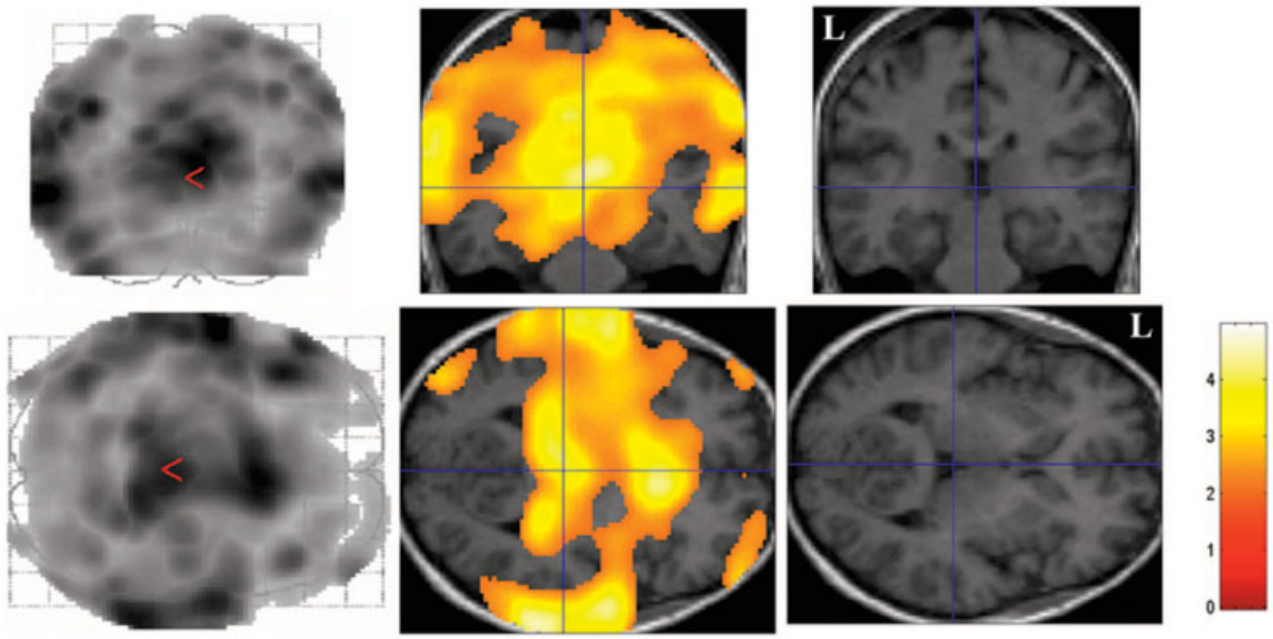

Fig. Brain areas with a significant decrease of [^{123}I]5-I-A-85380 distribution volume (V) *in Parkinson's disease patients detected with a two-sample* t *test in Statistical Parametric Mapping version '02. Areas with a significant decrease are displayed in the glass brain (left) and on transverse and coronal slices through thalamus of a magnetic resonance (MR) image of a control subject (middle). Corresponding MR images without superimposition are shown on the right. Highlighted areas showed* p *less than 0.05 false-discovery rate, which was corrected for multiple comparisons. The area also showed cluster-level corrected* p *less than 0.001. Color bar shows* T *values with the maximum value of 4.96. Note that the glass brain view displays decreases in the entire brain superimposed to anteroposterior (top left) or top-bottom (bottom left) views, whereas the MR images with the superimposition display decreases on single slices.*

gion. There was no significant regression between any clinical rating scores and *V*.

Discussion

In this study, we detected a significant and widespread decrease of nAChRs in early to moderately affected, nondemented PD patients by applying accurate quantification with the measurement of arterial input function and the plasma free fraction (f_1) of [^{123}I]5-I-A-85380 in each subject. Such measurements made the outcome of imaging studies free from intersubject and between-group differences in the metabolism and the protein binding of the imaging agent. Because patients tended to show lower f_1 values, if total plasma parent had been used instead of free [^{123}I]5-I-A-85380, the decrease in *V* would have been overestimated. Furthermore, for accurate measurement, scatter correction[15] and a pixel-based modeling that minimizes noise-induced biases[16,17] were applied.

There are three possible reasons that the decreases in *V* detected in this study were smaller than those reported at postmortem (>30% in most studies). First, because there is no large region devoid of nAChRs, it was not possible to measure nondisplaceable radioactivity, which then could have been used to calculate specific binding of [^{123}I]5-I-A-85380. Because *V* is a summation of specific and nondisplaceable distribution volumes, a decrease of specific binding was underestimated. Second, in this pilot study, only nondemented PD patients were enrolled, whereas postmortem studies found larger decreases in nAChRs in demented patients.[5,7] Therefore, postmortem studies including demented patients showed greater decreases in nAChR than in this study. The lack of significant regression between clinical ratings and *V* may also be explained by a fairly uniform population of nondemented patients. Third, whereas B_{max} is measured in postmortem studies, B_{max}/K_d plus nondisplaceable activity is measured by *in vivo* imaging studies including this one. If there were a decrease in K_d measured *in vivo* in addition to a decrease in B_{max}, a decrease in B_{max}/K_d would not be as great as that in B_{max}. In fact, a postmortem study reported a nonsignificant but substantial 10 to 40% decrease in K_d in both cortical and subcortical regions.[6]

There are a couple of factors that may confound interpretation of the results of this study. All patients were taking L-dopa–containing medications. L-Dopa treatment significantly decreased *in vitro* [^{125}I]5-I-A-85380 binding in the striatum, but not in cerebral cortex in normal squirrel monkeys.[19] However, in the same study, L-dopa treatment did not decrease [^{125}I]5-I-A-85380

binding in the same regions in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine–treated animals whose dopaminergic terminals were almost completely destroyed. Therefore, the widespread decreases in nAChRs found in this study are more likely to be the result of PD pathology than L-dopa treatment. Brain atrophy can cause widespread decrease in nAChRs detected in SPECT. However, a voxel-based morphometric study on nondemented patients did not detect a widespread decrease in gray matter volume.[20] By taking together the factors described earlier, nondemented patients with PD did show a widespread decrease of B_{max}/K_d in β_2-containing nAChRs both in cortices and subcortical regions. Because postmortem studies have shown greater decreases in nAChRs in demented patients, it would be interesting to extend the study to include such patients.

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